SUBSTANCES WITH JUVENILE HORMONE ACTIVITY IN CRUSTACEA AND OTHER INVERTEBRATES [1]

HOWARD A. SCHNEIDERMAN AND LAWRENCE I. GILBERT [2]

Department of Zoology, Cornell University, Ithaca, N. Y., and Marine Biological Laboratory, Woods Hole, Mass.

The cyclical growth and molting of immature insects is brought about by two hormones, one secreted by the insect's brain and the other by the prothoracic glands. A third hormone, the juvenile hormone, is secreted by the corpora allata, endocrine glands in the head or prothorax of the insect. This hormone promotes larval development but prevents metamorphosis (Wigglesworth, 1957). Its presence in the immature insect guarantees that when the larva molts it will retain its juvenile characters and not differentiate into an adult. The juvenile hormone is thus a remarkable molecule that permits growth but prevents maturation. So far as we are aware it has no functional counterpart in the vertebrates. Recently Williams (1956) has reported that ether extracts of the abdomens of male Cecropia moths (*Hyalophora cecropia* L.) contain large amounts of juvenile hormone. When this extract was injected into lepidopterous pupae, they molted into second pupae instead of molting into adults. This, of course, is precisely what occurs when active corpora allata are implanted into pupae (Piepho, 1951; Williams, 1952).

Although initial experiments demonstrated juvenile hormone only in extracts of male Cecropia moths, we have since extracted it from both males and females of 22 species of Lepidoptera representing 6 families (Schneiderman and Gilbert, 1957; Gilbert and Schneiderman, 1958a). This result suggested that the hormone could have a wider distribution in the animal kingdom. The experiments to be reported were conducted to determine whether substances with juvenile hormone activity could be extracted from other insect orders besides Lepidoptera, from other classes of arthropods and from other phyla.

Materials and Methods

1. *Experimental animals*

Pupae of the polyphemus silkworm (*Antheraea polyphemus* Cram.) were used as test-objects for assay of juvenile hormone activity. They were stored for about thirty weeks at 6° C. prior to use.

2. *Preparation and assay of extracts*

Animals representing most of the major groups of invertebrates were collected at Woods Hole, preserved in methanol and shipped to Cornell University for ex-

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[2] Present address: Department of Biology, Northwestern University, Evanston, Illinois.

traction. Some animals (*e.g.,* earthworms and slugs) were collected locally and extracted immediately. The tissues were homogenized in ethyl ether and the homogenates and methanolic extracts vigorously re-extracted with ether in a continuous extractor. The ether extracts were washed several times with water, the ether evaporated off and the oily or waxy residue dried *in vacuo* at 60° C.

To test for juvenile hormone activity in the resulting extracts, many of which were toxic and waxy, a new and exceedingly sensitive assay procedure was developed which permitted detection of traces of juvenile hormone activity in crude extracts. The assay takes advantage of the extraordinary sensitivity of regenerating epidermal tissue to juvenile hormone (Piepho, 1950; Piepho and Heims, 1952). The extract to be assayed is mixed with peanut oil and paraffin wax. A small rectangle of integument is excised from the thorax of a Polyphemus pupa, a few crystals of streptomycin and phenylthiourea (an anti-tyrosinase) placed in the wound, and the wound sealed with a few milligrams of melted wax-peanut oil-extract mixture. When the adult moth emerges three to four weeks later, the wound area is examined. In the case of inactive extracts, the only evidence of the former wound is a small indentation covered with adult cuticle. However, if the extract is active, then an island of pupal cuticle occurs at the wound site. Figure 1 depicts such a patch of pupal cuticle. It stands out sharply from the adult cuticle which surrounds it. It is scale-less, brown, rugose and typically pupal in

FIGURE 1. Thorax of adult Polyphemus with a pupal patch produced by the wax test. Scales have been removed.

Table I

Effects of serial dilutions of crude juvenile hormone extract in paraffin

Concentration of hormone in paraffin	Effect*		
0 (Peanut oil)	0	0	0
0 (Inactive oils)	0	0	0
0 (Paraffin)	0	0	0
1:2000	+	0	0
1:512	+	0	0
1:256	+	0	0
1:128	+	+	0
1:64	+	+	0
1:32	+	+	+
1:16	+	+	+
1:8	+	+	+
1:4	+	+	+
1:1	+	+	+

* Each symbol represents a test animal.

most other respects; it may even have pupal setae. In cross-section it appears to be three to four times as thick as adult thoracic cuticle. In short, it is essentially indistinguishable from ordinary pupal cuticle.

This "wax test" appears to be far more sensitive than other tests for juvenile hormone activity, as the following experiment reveals. A crude ether extract of the abdomens of male Cecropia moths was serially diluted with peanut oil (up to 1/1000) and these dilutions dissolved in equal parts of wax and applied to thoracic wounds as described above. The results recorded in Table I reveal that the assay permits detection of final dilutions of hormone of 1/2000. That is, in principle, an extract that contained only 1/1000th as much juvenile hormone activity as male Cecropia extract would yield a positive result. In the 1 2000 dilution recorded in Table I, the wax patch weighed about 6 milligrams and, therefore, contained only *3 micrograms* of crude extract. Hence it is possible with this test to assay the juvenile hormone content of minute quantities of material extracted from a part of a single insect.

It is important to note that, so far as we can ascertain, the wax test is absolutely specific for juvenile hormone activity. Thus wax alone, or mixtures of wax with peanut oil, have never given us false "positive tests" although dozens of control tests have been made. Moreover, when the active principle is removed from the crude extract by repeated liquid-liquid extractions, the oil that remains, containing virtually all of the ether-extractable material in the original extract, is also inactive in the wax test.

Results and Discussion

Using this sensitive test, extracts from 13 classes of invertebrates representing most of the major phyla were examined. The results presented in Table II reveal that ether extracts of a truly diverse array of invertebrates possess at least some juvenile hormone activity. It is not too surprising to find juvenile hormone activity in crustaceans and even in annelids, but surely its presence in hydroids and sea cucumbers is unexpected.

TABLE II

Juvenile hormone activity of ether extracts of various invertebrates

Phylum	Class	Species	Wax test
Porifera	Demospongiae	*Microciona prolifera*	0
		Cliona celata	0
Cnidaria	Hydrozoa	*Pennaria tiarella*	+
		Tubularia crocea	+
	Anthozoa	*Metridium dianthus*	0
Rhynchocoela	Anopla	*Cerebratulus* sp.	
		(bodies)	0
		(heads)	0
Annelida	Polychaeta	*Nereis virens*	
		(bodies)	+
		(heads)	+
	Oligochaeta	*Lumbricus terrestris*	
		(bodies)	+
		(heads)	+
Arthropoda	Insecta	Numerous Lepidoptera	+
		Tenebrio molitor (Coleoptera)	
		(larvae)*	+
		(adults)	0
		Sarcophaga bullata (Diptera)	
		(larvae)	0
		Neodiprion lecontei (Hymenoptera)	
		(diapausing prepupae)	0
		Apis mellifera (Hymenoptera)	
		(winter workers)	0
	Crustacea	*Uca pugilator*	+
	(Decapoda)	*Orconectes immunis*	
		(entire)	0
		(purified extract)	0
		Homarus americanus	
		(eyestalks)	+
		Carcinides maenas	
		(fronts)	0
		(rears)	0
		Palaemonetes vulgaris	0
	Arachnida	*Limulus polyphemus*	
		(fronts)	0
		(rears)	0
		(purified sterols)	0
Mollusca	Gastropoda	*Deroceras* (*Agriolimax*) *agreste*	
		(heads)	0
Echinodermata	Holothuroidea	*Thyone briareus*	0
		Leptosynapta inhaerens	+
	Echinoidea	*Arbacia punctulata*	0
Enteropneusta	Balanoglossida	*Saccoglossus kowalevskii*	
		(entire)	+
		(less collar and proboscis)	+

* Tested by injecting extract.

It is of some interest that the most potent non-insect extract came from the eyestalks of lobsters. The occurrence of high concentrations of substances with juvenile hormone activity in the eyestalk, which is a well-known endocrine center

in crustaceans (Knowles and Carlisle, 1956), suggests that in crustaceans the eyestalk may contain a gland which produces a substance chemically similar to the juvenile hormone of the corpora allata. A likely site is a part of the X-organ which is not neurosecretory but appears glandular (*i.e.*, the secretory cells of the sensory papilla X-organ (Knowles and Carlisle, 1956)). Whether the juvenile hormone plays a role in crustacean development or egg maturation remains to be proved, but it appears likely. In addition to these results we have also recently found juvenile hormone activity in the adrenal cortex of cattle (Gilbert and Schneiderman, 1958b). Hence, it seems safe to conclude that substances with juvenile hormone activity are widespread in the animal kingdom. As far as we are aware, the only other animal growth hormones of such wide distribution are the estrogens (Loewe *et al.*, 1932; Hagerman *et al.*, 1957).

Whether or not these juvenile hormone substances are similar chemically to the juvenile hormone of insects cannot be answered until the structure of the juvenile hormone is known, nor do we know at present what role these juvenile hormone substances play in groups other than insects. Nevertheless, it remains an intriguing fact that substances that act as a growth hormone for insects occur in both hydroids and cattle. It supports the view that in the course of evolution there have not been a great number of innovations at the level of small molecules since the Cambrian Era, and that the evolution of humoral mechanisms has proceeded by particular groups of animals adapting available and often ubiquitous molecules to special tasks.

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Summary

1. A new assay for the juvenile hormone of insects is described which permits detection of very small amounts of hormone activity.

2. Using this procedure extracts of a variety of invertebrates were assayed for juvenile hormone activity.

3. Juvenile hormone activity was detected in Hydrozoa, Polychaeta, Oligochaeta, Lepidoptera, Coleoptera, Decapoda, Holothuroidea, and Balanoglossida.

4. The richest source of juvenile hormone outside of insects was the eyestalk of Crustacea and it is suggested that the juvenile hormone plays a role in crustacean physiology.

5. The significance of these findings is discussed in relation to the evolution of humoral mechanisms.

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